

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. Serial No.: 09/431,451

Group Art Unit: 1650

Filing Date: November 1, 1999

Examiner: Sisson, B.

Applicant: Senapathy, P.

Attorney Docket No.: 34623.005

Title: METHOD FOR AMPLIFYING SEQUENCES FROM UNKNOWN DNA

FULL SET OF "CLEAN" CLAIMS AS AMENDED, 37 CFR §1.121(c)(3)

Sub F1
E1
X **[AMENDED THREE TIMES]** A method of amplifying desired regions of nucleic acid from a nucleic acid template comprising:

- (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;
- (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
- (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the template at locations removed from the first primers such that nucleic acid regions flanked by the first primer and the second primer are specifically amplified.

- 2. [AMENDED] The method of Claim 1, wherein the template is genomic DNA.
- 3. [AMENDED] The method of Claim 1, wherein the template is eukaryotic genomic DNA.
- 4. [AMENDED] The method of Claim 1, wherein template is human genomic DNA.
- 5. [AMENDED] The method of Claim 1, wherein the template is prokaryotic DNA.

6. [AMENDED] The method of Claim 1, wherein the template is DNA selected from the group consisting of cloned genomic DNA, a subgenomic region of DNA, a chromosome, and a subchromosomal region.
7. [AMENDED] The method of Claim 1, wherein the template is RNA.
8. The method of Claim 1, wherein in step (a) is provided a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence complementary to a consensus sequence selected from the group consisting of a promoter sequence, a 3' splice sequence, a 5' splice sequence, an Alu repeat, a tandem repeat, poly-A site, a lariat signal, a microsatellite sequence, and a homeobox sequence.
9. The method of Claim 1, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.
10. The method of Claim 1, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of over 50%, under 50%, and about 50%, and in step (b) is provided a plurality of second primers having a G+C content selected from the group consisting of over 50%, under 50%, and about 50%.
11. The method of Claim 1, further comprising step (d): incorporating the amplified fragments of step (c) into a library.

12. **[AMENDED THREE TIMES]** A method of amplifying exons from a nucleic acid template comprising:

- Sub F2
- EP
- (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of a 3' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;
 - (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence reversely complementary to a consensus sequence of a 5' splice region and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
 - (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to a sequence reversely complementary to the 3' splice consensus sequence substantially wherever it occurs in the template, and a subset of the plurality of second primers binds to the 5' splice consensus sequence substantially wherever it occurs in the

template, such that exons flanked by the first primer and the second primer are specifically amplified.

13. The method of Claim 12, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.
14. The method of Claim 12, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of cover 50%, under 50%, and at 50%, and in step (b) is provided a plurality of second primers having a G+C content selected from the group consisting of cover 50%, under 50%, and at 50%.
15. The method of Claim 12, further comprising step (d): incorporating the amplified fragments of step (c) into a library.
16. [AMENDED] The method of Claim 12, wherein a genomic DNA template is amplified.
17. [AMENDED] The method of Claim 12, wherein a human genomic DNA template is [specifically] amplified.
18. [AMENDED] The method of Claim 12, wherein a DNA template selected from the group consisting of cloned genomic DNA, a subgenomic region of DNA, a chromosome, and a subchromosomal region is amplified.

- Sub
E3
19. **[AMENDED THREE TIMES]** A method of amplifying regions flanking a consensus sequence in a nucleic acid template of totally or partially unknown sequence comprising:
- (a) providing a plurality of first PCR primers, each first primer having an overall length of at least about 10 nucleotides and further having a region of fixed nucleotide sequence identical or complementary to a consensus sequence of interest and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence;
 - (b) providing a plurality of second PCR primers, each second primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; then
 - (c) amplifying the nucleic acid template via the PCR using the plurality of first PCR primers and the plurality of second PCR primers under conditions wherein a subset of the plurality first primers binds to the consensus sequence of interest substantially wherever it occurs in the template, and a subset of the plurality of

second primers binds to the template at locations removed from the first primers such that nucleic acid regions flanked by the first primer and the second primer are specifically amplified; then

- (d) incorporating the amplified nucleic acid of step (c) into a library;
- (e) sequencing a portion of amplified nucleic acid from a particular clone from the library of step (d) and providing a third PCR primer of unique sequence and having an overall length of at least about 10 nucleotides which will prime PCR amplification from the sequenced portion of DNA;
- (f) providing a plurality of fourth PCR primers, each fourth primer having an overall length of at least about 10 nucleotides and further having a region of arbitrary, yet fixed nucleotide sequence and a region of randomized nucleotide sequence located 5' to, 3' to, or flanking the region of fixed nucleotide sequence; and then
- (g) amplifying the nucleic acid present in the template via the PCR using the third PCR primer and the plurality of fourth PCR primers under conditions wherein the third primer binds to the sequenced portion of nucleic acid from step (e), and a subset of the plurality of fourth primers binds to the template at locations removed from the third primers such that nucleic acid regions flanked by the third primer and the fourth primer are specifically amplified.

20. [AMENDED] The method of Claim 19, wherein the template is genomic DNA.
21. [AMENDED] The method of Claim 19, wherein the template is eukaryotic genomic DNA.
22. [AMENDED] The method of Claim 19, wherein the template is human genomic DNA.
23. [AMENDED] The method of Claim 19, wherein the template is prokaryotic DNA.
24. [AMENDED] The method of Claim 19, wherein the template is DNA selected from the group consisting of cloned genomic DNA, a subgenomic region of DNA, a chromosome, and a subchromosomal region.
25. [AMENDED] The method of Claim 19, wherein the template is RNA.
26. The method of Claim 19, wherein in step (a) is provided a plurality of first PCR primers, each first primer having a region of fixed nucleotide sequence identical or complementary to a consensus sequence selected from the group consisting of a promoter sequence, a 3' splice sequence, a 5' splice sequence, an Alu repeat, a tandem repeat, poly-A site, a lariat signal, a microsatellite, and a homeobox sequence.
27. The method of Claim 19, wherein in step (a) is provided a plurality of first primers having an overall length of from about 10 nucleotides to about 30 nucleotides, and in

step (b) is provided a plurality of second primers having an overall length of from about 10 nucleotides to about 30 nucleotides.

28. The method of Claim 19, wherein in step (a) is provided a plurality of first primers having a G+C content selected from the group consisting of cover 50 %, under 50 %, and at 50 %, and in step (b) is provided a plurality of second primers having a GC content selected from the group consisting of cover 50 %, under 50 %, and at 50 %.
29. The method of Claim 19, further comprising step (h): incorporating the specifically amplified fragments of step (g) into a library.